DOCUMENT-IDENTIFIER: US 20080166705 A1
TITLE: OPTOELECTRONIC DETECTION SYSTEM

Description of Disclosure:
[0084]FIG. 51 is a graph depicting the results of antibody 6E10-10, crosslinked to Protein G magnetic beads, which was incubated with varying amounts of **BoNT**/A Hc for 3 hours at 4.degree. C. Beads were washed with CO2I medium three times. Emittor cells expressing 6B2-2 antibody were added, the reaction was spun for 5 seconds, and the light output was monitored in a luminometer.

Description of Disclosure:
[0101]Alternatively, the cell can be a fibroblast. However, fibroblasts do not contain the signal transduction machinery necessary to transfer a signal from the cytoplasmic portion of a surface antibody to calcium stores in the cell. To overcome this problem, a chimeric surface antibody can be expressed in the fibroblast. This chimeric antibody contains a cytoplasmic amino acid sequence derived from a polypeptide (e.g., a **fibroblast growth factor receptor**) that can transduce a signal from the inner surface of the plasma membrane of the fibroblast to intracellular calcium stores. Thus, when an antigen binds to the extracellular portion of the chimeric antibody to cause antibody aggregation on the surface, calcium mobilization is induced. A similar strategy using chimeric antibodies can be employed for any other cell type which is not a B cell, so that the cell is suitable for use in the devices and methods of the invention.

Description of Disclosure:
[0102]Cells useful in the devices and methods herein are those designed to recognize a specific substance, including those having receptors on their surface that specifically bind to that substance. A preferred receptor is an antibody or single-chain antibody, although other suitable receptors include a mitogen receptor (such as a lipopolysaccharide (LPS) receptor), a macrophage scavenger receptor, a T cell receptor, a cell adhesion molecule, a DNA binding protein such as part of a sequence-specific restriction enzyme or transcription factor, single-stranded-RNA- or double-stranded-RNA-binding protein, an oligonucleotide complementary to a DNA or RNA sequence to be recognized, or other ligand-binding receptor (e.g., Fas; cytokine, interleukin, or hormone receptors; neurotransmitter receptors; odorant receptors; chemoattractant receptors, etc.) that will specifically bind the substance to be recognized. The receptor can be attached to the cell surface via a transmembrane domain, a membrane-bound molecule that specifically binds to the receptor (such as Fc receptors bind to antibodies), or a covalent or noncovalent attachment (e.g., biotin-streptavidin, disulfide bonds, etc.) to a membrane-bound molecule. The receptor can also be a chimeric molecule; for instance, it can have an extracellular domain such as an antibody, single-chain antibody, lectin or other substance-specific binding domain or peptide, and an intracellular domain such as that from the insulin **receptor, fibroblast growth factor,** other protein that triggers a second messenger cascade, etc. Instead of directly binding to the substance to be recognized, the receptor might specifically bind to another molecule or object that in turn specifically binds to the substance to be recognized, such as a secondary antibody, labelled bead, antigen-conjugated oligonucleotide, etc.

Description of Disclosure:
[0112]After the desired member of the library is identified, the specific sequence can be cloned into any suitable nucleic acid expressor (e.g., a vector) and transfected into a cell such as a fibroblast. The expressor can also encode amino acids operably linked to the antibody sequence as appropriate for the cell which is to express the antibody. As discussed above, the cytoplasmic transmembrane sequence of a **fibroblast growth factor receptor** can be linked to a single-chain antibody specific for the antigen to be detected, so that the cell immobilizes calcium when contacted with the antigen. Although separate recombinant heavy chains and light chains can be expressed in the fibroblasts to form the chimeric

antibody, single chain antibodies also are suitable (see, e.g., Bird et al., Trends Biotechnol 9:132-137, 1991; and Huston et al., Int Rev Immunol 10:195-217, 1993).

Description of Disclosure:

[0234]This second method has been demonstrated in practice, using the heavy chain of botulinum toxin type A (BoNT/A Hc) as the soluble, monomeric target protein (FIG. 51) and antibodies described in Pless et al., Infection and Immunity (2001) 570-574. Monoclonal antibody (6E10-10) against one epitope was crosslinked to protein G-coated beads. These beads were incubated with BoNT/A Hc for 3 hrs at 4.degree. C., washed, and used to stimulate emittor cells expressing a second antibody (6B2-2) that recognizes a different BoNT/A Hc epitope. The BoNT/A Hc-decorated beads effectively stimulated the emittor cells, with an LOD of about 6 ng. Emittor cells expressing the same antibody as that used to bind the BoNT/A to the beads were not stimulated, indicating that the emittor reaction was not caused by aggregation of the target protein.